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14. ABSTRACT
Approximately 10%-20% of individuals serving in Iraq and Afghanistan have Posttraumatic Stress Disorder (PTSD). Women serving in the military have been shown to be twice as likely to develop PTSD in their lifetime compared to men.1-3 Studies aimed at identifying vulnerability factors for women serving in the current operations (OEF/OIF) are of significant public health concern as the US has witnessed a significant increase of women in the US military in the past decade, among who now comprise the largest cohort of female veterans (11.4%). Relatively little is known about gender differences among women deployed to OEF/OIF4. In order to address this critical gap we will examine 40 OIF/OEF female PTSD positive cases and 40 OEF/OIF female PTSD negative control subjects through an extensive biological protocol as a supplement to the DOD funded comprehensive Biomarkers for PTSD study.

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INTRODUCTION:

There is a growing body of research that reports high rates of PTSD in those who have served in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (Afghanistan; OEF), with estimates varying from 10% to 20%[1]. An important limitation is the reliance on self-report screening measures and clinical interviews to make the diagnosis of PTSD. In sharp distinction with medical disorders such as cancer, coronary artery disease and diabetes for which there are objective biomarkers for diagnosis, illness severity, and response to treatment, the assessment of PTSD cannot be independently confirmed by biological markers.

Among one of the most pressing challenges the US will face from current combat operations in Iraq and Afghanistan will be the mental health burdens placed on women serving in these operations, and in particular the negative impact of PTSD. Studies consistently find that although men are at greater risk for exposure to potentially traumatic events, women are twice as likely to develop PTSD in their lifetime ([10.4 vs. 5.0%] [2-6] including military samples.[7-9]

Studies aimed at identifying vulnerability factors for women serving in the current operations (OEF/OIF) are of significant public health concern as the US has witnessed a significant increase of women in the US military in the past decade, accounting for 15% of active duty and 17% of National Guard and Reserve personnel, among whom now comprise the largest cohort of female veterans (11.4%). In contrast to previous operations, women deployed to OEF/OIF appear to be at greater risk for trauma exposure as they are now being deployed for longer periods of time in settings in which the front-lines are more ambiguous[10-11].

It has been suggested that biological mechanisms may explain the observed gender differences in PTSD[12-15]. Discovering biomarkers for PTSD in women would aid, not only in more accurate diagnoses, but in elucidating biological pathways that increase the risk for PTSD and enable more targeted forms of treatment. The identification of a biomarker for PTSD in women would also aid in staging the course of the illness, including identifying pre-morbid biological dysregulation, which might enable earlier treatment. If the biomarkers under study prove competent in objectively tracking disease severity, this could also lead to developing new metrics by which the risk of, and recovery from, PTSD could be gauged, in PTSD in general, and among women in particular[12-15].

In order to address this critical gap we will examine 40 OIF/OEF female PTSD positive cases and 40 OEF/OIF female PTSD negative control subjects through an extensive biological protocol as a supplement to the DOD funded comprehensive Biomarkers for PTSD study.

BODY:

Accomplishments During this Reporting Period (10/23/12- 10/22/13)

The Biomarkers for PTSD in Female Iraq and Afghanistan Veterans study is in the implementation phase. In the second year of the grant we accomplished several milestones and goals. These accomplishments are detailed below:

1. Communication Strategies

The team continued to engage in bi-weekly communication meetings via teleconference to ensure the successful and timely execution of the Implementation Phase.

Calls took place between the PIs and investigators at each site (SF VAMC, UCSF, Emory University, Mt Sinai, Bronx VA and NYU). Meetings addressed safety issues, clinical questions, strategies for improving subject recruitment and enrollment, strategies for maximizing participation in Visits 2-4, and ensuring that participants moved through all stages of the study quickly and efficiently (in order to avoid attrition).

The clinical team, under the supervision of Dr. Henn-Haase, conducted weekly calibration meetings across sites to establish clinical consensus in scoring the frequency and intensity of symptoms on the CAPS and clinical assessment. Each discrepancy from the evaluation of participants was resolved by group consensus during these meetings.

2. Institutional Review Board (IRB)

Female subject recruitment has been challenging due to the differences in combat exposure and involvement that female veterans face compared to male veterans (i.e. females do not participate in direct combat). In order to increase enrollment of female veterans, we submitted an amendment to the IRBs at NYU, Bronx VA and MSSM to expand our inclusion criteria. Instead of only including female veterans with Civilian Administered PTSD Scale (CAPS) scores ≥ 40, we expanded the inclusion criteria to include female subjects who have a current CAPS score ≥ 30, and endorsed at least the minimum number of items in Cluster B and Cluster C or D on the CAPS. The proposed changes have been approved by the IRBs at all sites and were verbally approved by Karen Eaton, Human Subjects Protection Scientist of the Department of Defense (DOD). The amended IRB application was reviewed by the IRB full board.

We submitted an application to the NYU IRB for the annual continuation review and the application for continuation was approved on October 16, 2013.

3. Outreach and Recruitment Activities

The study flyer was re-designed and we refined the text under the "Eligibility" section to improve comprehension and clarity. Our outreach staff participated in the following events and meetings to recruit participants for our research study. Flyers and brochures advertising the research study were distributed

- SAMSHA meeting (Tuesday, April 3)
- Institute for Community Living Veteran Resource Fair (Wednesday, April 3)
- SWAN meeting (Thursday, April 4)
- Veterans Mental Health Coalition Meeting (April 5th, 10AM-2PM)
- NYU Military Veterans Club Luncheon (April 5th, 2PM-3PM)
- Columbia MilVets Ball (April 5th, evening)
- SSVF meeting (April 8th)
- Women in Combat Lecture @ NYU (Wednesday, April 17)
- Special guest speaker at Touro College for Veterans class (April 18th)
- MOVA event downtown (April 22nd)
- Tom Murphy's Edge 4 Vets program (May 3rd)
- Words of War Event with Weill Cornell & HeadStrong (Wednesday, May 8)
- Dr. Marmar's grand rounds at Brooklyn VA (May 10th)
- Veteran Civilian Dialogue: Moral Injury (Friday, May 10)
- Bronx Veterans' Appreciation Day Breakfast with Ruben Diaz (Monday, May 13)
- Women in the Military: Unseen Battles Panel @ Manhattan VA (Wednesday, May 22)
- Mayor's Office of Veterans Affairs Employment Fair (Tuesday, May 28)
- Meeting at Manhattan VA Mental Hygiene Clinic (May 29th)
- Veterans Mental Health Coalition Meeting (Friday, June 7)
- Stand Up For Women Veterans @ Westchester Community College (Friday, June 7)
- Veteran/Civilian Dialogue (Friday, June 7)
- Army Birthday Celebration – 4th Infantry Division's Family Picnic Day (Friday, June 14)
- Manhattan Single Stop Veterans Resource Fair (Tuesday, June 18)
- Manhattan VA PTSD Clinic Meeting (Wednesday, June 26)

4. Recruitment of Research Participants, Enrollment and Acquisition of Biomarkers

According to the Statement of Work, the goal of this grant is to enroll 80 OIF/OEF female veterans. During the second year of the grant, 120 participants were screened via telephone to determine initial eligibility for enrollment. Of those veterans, 94 provided consent and were enrolled in the study and completed the baseline clinical assessment. Of the 94 enrolled participants, 29 met all study criteria and were eligible for ascertainment of biomarkers.

Of those 29 eligible participants 11 were positive for PTSD and 18 were negative for PTSD. To date 25 participants successfully completed the blood study procedures, the self-report questionnaire and 21 completed the MRI and Neurocognitive testing.

All MRI scans were processed through FreeSurfer v5.1. Manual Hippocampal Subfield Markings were conducted on selected scans.

Participants were reimbursed for their time and effort after completing study procedures.

5. Standard Operating Procedure (SOP) Manuals & Procedure Manual for Handling of Samples

Our research operations team met many milestones during this reporting period.
All cores including those who are collecting specimens and acquiring and processing the MRI scans developed detailed SOP manuals for all study procedures. We anticipate that the finalized SOPs will be stored in the Datacube.

6. Data Management

All Clinical Assessment data from the baseline interview, self-report and neurocognitive measures for all study participants were completed and entered as digital data directly into the study secure SQL database server. Data from all cores is also shared with NYU and saved into a single centralized database.

We identified a sample of 10 cases and 10 controls that is matched on ethnicity and age and we will begin data analysis on this sample and circulate the data to the cores next month.

We conducted preliminary analysis on the demographical characteristics of 27 eligible Iraq and Afghanistan female veterans in the study. Results of this analysis are listed in tables below:

The Race/Ethnicity distribution of female is shown in the table below

Race/ethnicity	Frequency	Percent
Hispanic	8	29.63
Non-Hispanic Black	10	37.04
Non-Hispanic Other	3	11.11
Non-Hispanic white	6	22.22

The summary statistics of age in females is shown below

Analysis Variable : age				
N	Mean	Standard Deviation	Minimum	Maximum
27	31.59	5.57	24	49

The education level of female is shown in the table below

Education Level	Frequency	Percent
up to 12th grade	1	3.7
High school diploma	7	25.93
2 years college A.A. degree	8	29.63
4 years college Bachelor's Degree	8	29.63
Master degree	2	7.41
Doctoral Degree	1	3.7

The military branch information of female is shown in the table below

Military branch	Frequency	Percent
Air Force	2	7.69
Army	10	38.46
Marine	2	7.69
National Guard	3	11.54
Navy	4	15.38
Reserve	5	19.23

7. Shipment of Material to cores

Shipments of Blood samples were transferred to all collaborating sites, Integrative Systems Biology: Mouse Models of PTSD (Principal Investigator Dr. Jett), and Institute for Systems Biology (ISB): Genetics, Metabolomics (Principal Investigator Dr. Hood), Genetics Core at Emory University (PI: Dr. Kerry Ressler), and to the Metabolism Core at UCSF (PI: Dr. Owen Wolkowitz).

Data transfer from NYU to the imaging core at UCSF is running smoothly and Q & A procedures indicate high quality of data collection.

8. Request for No Cost Extension

Due to the complexity of recruitment of Iraq and Afghanistan female veterans and the acquisition of all the biomarkers we do not expect to accomplish all the milestones listed on the statement of work and the recruitment goals within this period. As a result, on October 7th we submitted a request for a one year extension to complete this work. No additional funds were requested since we have sufficient funds to complete this work.

KEY RESEARCH ACCOMPLISHMENTS:

- Refined recruitment materials for the study.
- Obtained IRB approvals for the continuation process across all sites and submitted to the DOD for final review.
- Conducted targeted outreach and networking with various veterans and community organizations. IRB approved recruitment material (brochures, flyers and advertisements) were distributed at job fairs, colleges, female clinics, VA Medical Centers and female veterans' organizations.
- Enrolled 94 OIF/OEF female veterans and completed clinical assessments. Of those 29 were found eligible and met study criteria.
- Study team from all sites participated in bi-weekly study meetings
- Entered, cleaned all data into a centralized database and ran reports and queries for tracking progress.
- Completed biomarkers study procedures on 25 eligible participants including blood draws, MRIs and urine collection.
- Completed shipments of blood samples from JJPVAMC to all cores including Metabolism, Multi-omics and Genetics cores. Neuroimaging data was transferred successfully from NYU center of brain imaging to UCSF.
- Developed Standard Operating Procedures Manuals (SOPs) and will store this in the datacube.

REPORTABLE OUTCOMES:

- The major development during the timeframe of this annual report for this project is that all cores are still active and working on the implementation phase.
- Recruitment and data collection is being accelerated and participants are completing all study procedures.
- Data and samples are being transferred across sites and several specimen shipments were sent to the metabolism, multi-omics and genetics cores.
- We submitted a request for a one year no cost extension to finish this work and accomplish the milestones listed in the statement of work.
- Tasks to complete for the next reporting period include:
    - (1) Continue to recruit and enroll subjects for the study.
    - (2) Run study participants through all procedures.
    - (3) Continue data collection and data management.
    - (4) Analyze demographic data for enrolled participants for the purpose of matching controls with PTSD positive participants.
    - (5) Continue to process and ascertain biomarkers.
    - (6) Test biomarkers for 20 cases/20 controls (Discovery Phase).

- (7) Replicate the most promising biomarkers in the next 20 cases/20 controls (Replication phase).
- (8) Continue to ship samples to UCSF, Emory University, Drs. Marti Jett and Lee Hood for analysis.
- (9) Develop an analysis strategy to disseminate results.

CONCLUSION:

The study has received IRB approval from recruiting sites to include Iraq and Afghanistan female veterans with CAPS score > 30, which will allow us to accelerate the recruitment process . Recruitment material for the study was refined and the team started targeted outreach effort to recruit female OIF/OFE veterans. We completed baseline clinical interviews on 94 female veterans. Acquisition of biomarkers was completed on those who met the study criteria and biological samples were shipped to the collaborating sites. Data collection and data management is running smoothly. All data is maintained in a centralized data base at NYU.

We will start first batch of data analysis with a sample size of 10 cases/10 controls is achieved. The most promising biomarkers will be replicated and compared with female mouse model.

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